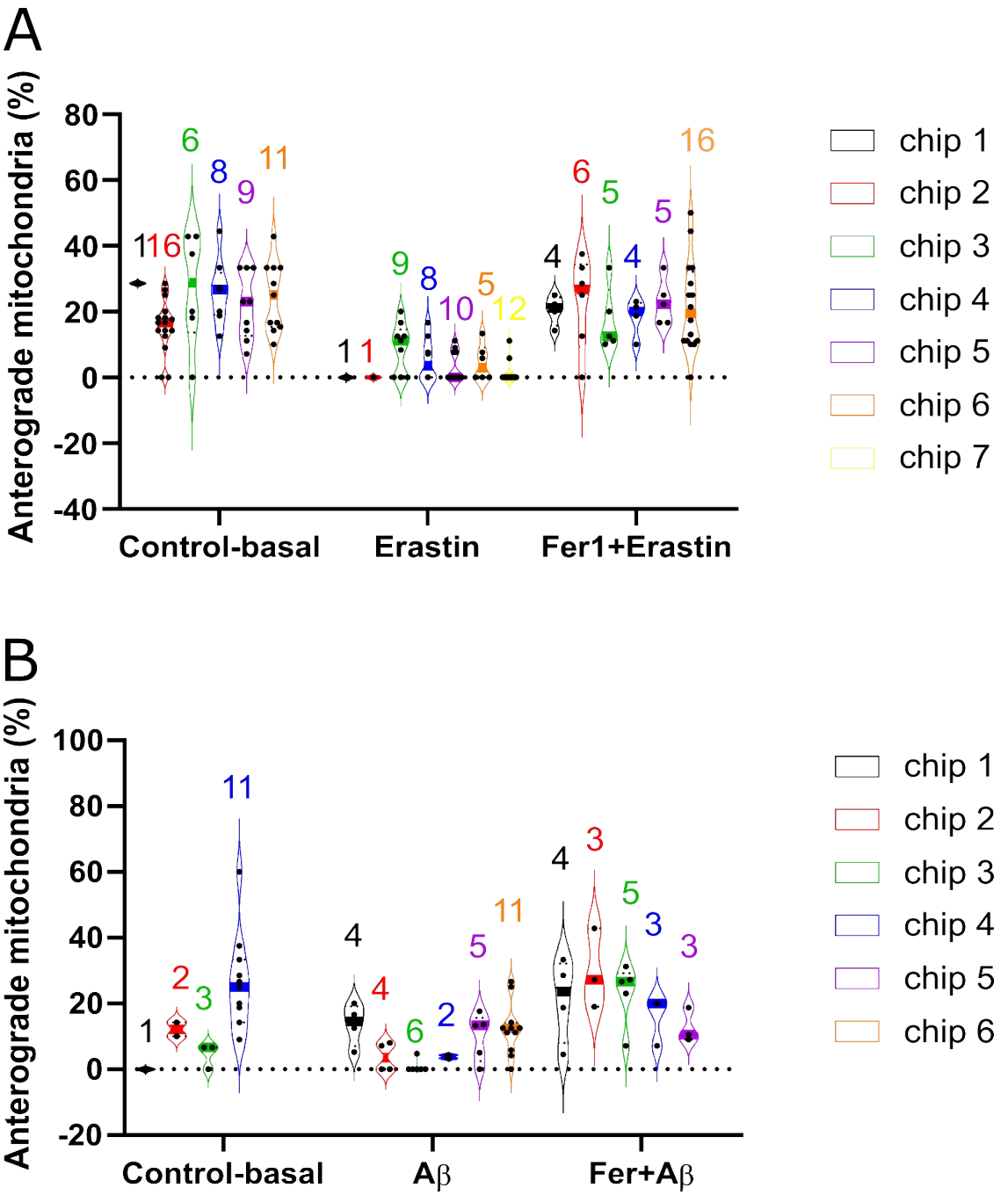
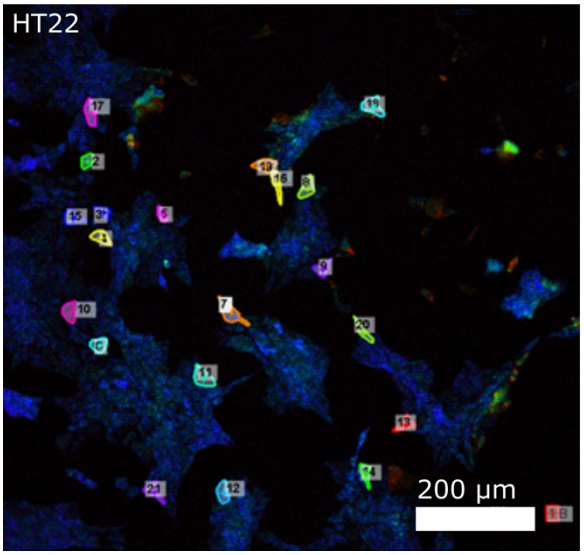
Supplementary material

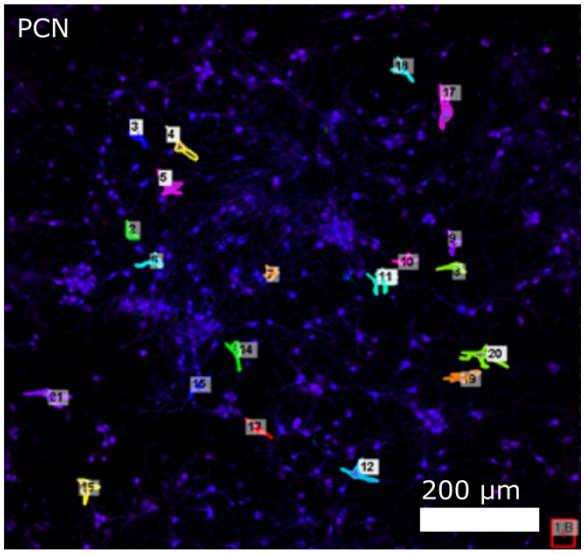


Supplementary Figure 1: Summary of microfluidic chambers and axons analyzed per experimental condition. *(A) Number of independent microfluidic chambers (chips) and axons analyzed in experiments in which chambers were treated with Erastin and the ferroptosis inhibitor Ferrostatin-1. (B) Number of independent microfluidic chambers (chips) and axons analyzed in experiments in which chambers were treated with Amyloid-beta in the presence or absence of Ferrostatin-1.*

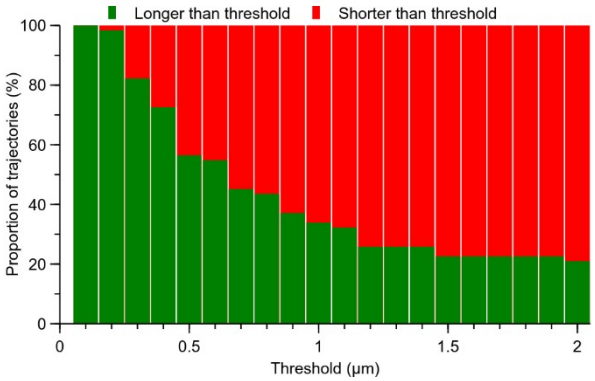
A



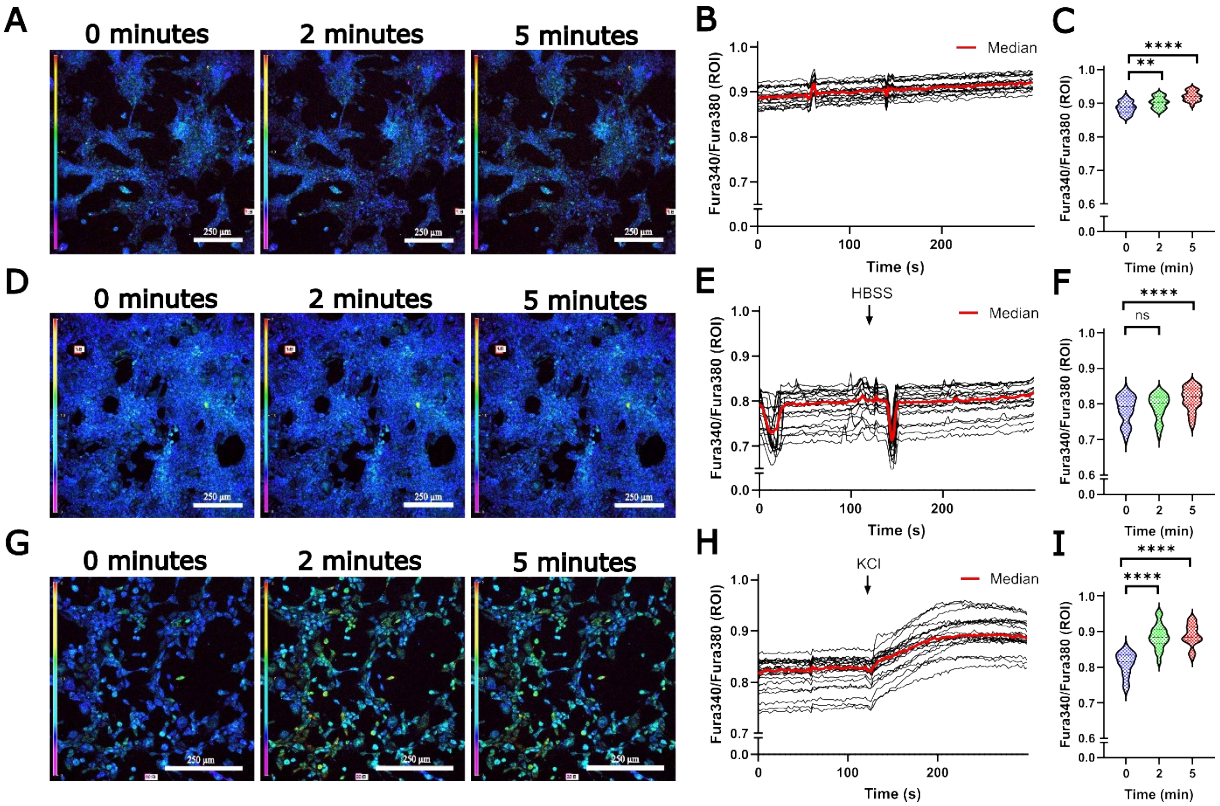
B



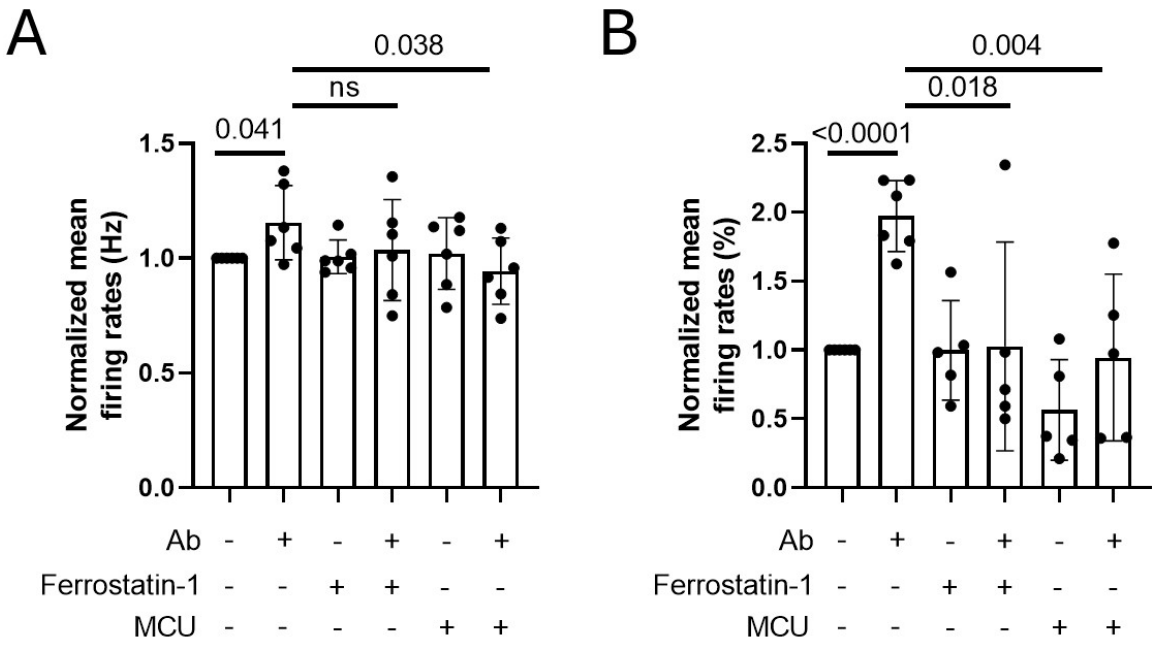
Supplementary figure 2: Cell selection in baseline measurement in HT22 cells and PCNs. (A) *Selection of HT22 cells for mt-fura-2 ratio measurement. The fura340/fura380 ratio of 20 randomly selected cells was measured for 5 minutes. Corresponds to Figure 5H and 6G.*



Supplementary figure 3: Proportion of far-moving mitochondria as a function of the distance defining what "far" means for mitochondria in primary neurons seeded onto glass. *For a given threshold distance, the displacement of each tracked mitochondrion was calculated and subsequently it was determined whether the mitochondrion moved further than the threshold distance (green) or not (red). The results for a threshold of 2 µm are the same as presented in Fig. 1D.*



Supplementary figure 4: Mitochondrial calcium imaging using mt-fura-2 in HT22 cells. *HT22 cells were stained with mt-fura-2 and the ratio of fura340/fura380 was measured for 5min. (A) representative image of ratio at 0, 2 and 5min. (B) a graph representing the ratio of fura340/fura380 with each line being one measured cell, and median highlighted in red. (C) Statistical analysis of the difference in the levels of the fura340/fura380 ratio between 0, 2 and 5min time point. (D-I) Baseline was measured for 2 minutes, followed by the treatment, which was measured for 3 minutes. (D-F) HT22 cells were treated with HBSS which had no acute effect on the fura340/fura380 ratio and (G-I) KCl (100 mM) which induced an acute increase of the fura340/fura380 ratio ($p<0.0001$) ($n=3$).*



Supplementary figure 5: Normalized mean firing rates in the presence and absence of Aβ1-42 with Ferrostatin-1 and MCU-i4 treatments. *(A, B) Two additional replicates of experiment shown in figure 6 in main text. The spiking rate increased significantly in the presence of Aβ. MCU-i4 significantly reduced the Aβ1-42-induced firing rate, whereas Fer-1 had no effect. Data are presented as mean±SD. Statistical difference was calculated with an unpaired t-test.*

Supplementary video 1: Example time-lapse video of a microfluidic chip axonal compartment labelled with MitoTracker (in red), showing mitochondrial movement in an axon exiting the microchannels (on the left) upon acute treatment with erastin (30uM) and with ferrostatin (1uM) after ferrostatin pretreatment (1h). *Motile mitochondria can be observed moving both to the left and to the right, corresponding to anterograde and retrograde transport, respectively. Movement toward the left indicates trafficking toward the soma, whereas movement toward the right reflects movement toward the distal axonal extremities.*